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Validation Of Cell-Cycle Arrest Biomarkers For Acute Kidney Injury Using Clinical Adjudication

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At a Glance Commentary:

Scientific Knowledge on the Subject

Through the systematic search of PubMed and Cochrane Library we identified 18 prospective studies, one meta-analysis and five recent in-depth reviews of urinary biomarkers of acute kidney injury in critically ill patients, published before January, 2014 (additional detail provided in the Supplement). Only one study prospectively compared all biomarkers against each other and found that the biomarker panel [TIMP-2]•[IGFBP7] had the highest area under the receiver-operating characteristics curve for AKI. No prior study has prospective validated biomarker cutoffs nor used clinical adjudication as the gold standard for the endpoint.

What This Study Adds to the Field

This is the first report of a multi-center biomarker study that prospectively validates a pre-defined biomarker cutoff value for risk assessment of acute kidney injury where the endpoint was determined by clinical adjudication of experts blinded to the results of the test. The urinary [TIMP-2]•[IGFBP7] test significantly improves risk assessment by stratifying patients into distinct risk categories, with a 7-fold increase in risk for patients with a [TIMP-2]•[IGFBP7] test value above the cutoff compared to those at or below. Furthermore, the [TIMP-2]•[IGFBP7] test provides substantial new information not available from the clinical variables known to be associated with AKI.

This article has an online data supplement, which is accessible from this issues's table of contents online at www.atsjournals.org.

Abstract

Rationale. We recently reported two novel biomarkers for acute kidney injury (AKI), tissue inhibitor of metalloproteinases (TIMP)-2 and insulin-like growth factor binding protein 7 (IGFBP7), both related to G1 cell cycle arrest.

Objectives. We now validate a clinical test for urinary [TIMP-2]•[IGFBP7] at a high-sensitivity cutoff > 0.3 for AKI risk stratification in a diverse population of critically ill patients.

Methods. We conducted a prospective multicenter study of 420 critically ill patients. The primary analysis was the ability of urinary [TIMP-2]•[IGFBP7] to predict moderate to severe AKI within 12 hours. AKI was adjudicated by a committee of three independent expert nephrologists who were blinded to the results of the test.

Measurements. Urinary TIMP-2 and IGFBP7 were measured using a clinical immunoassay platform.

Main Results. The primary endpoint was reached in 17% of patients. For a single urinary [TIMP-2]•[IGFBP7] test, sensitivity at the pre-specified high-sensitivity cutoff of 0.3 $(ng/ml)^2/1000$ was 92% (95% CI 85%-98%) with a negative likelihood ratio of 0.18 (95% CI 0.06-0.33). Critically ill patients with urinary [TIMP-2]•[IGFBP7] > 0.3 had seven times the risk for AKI (95% CI 4-22) compared to critically ill patients with a test result below 0.3. In a multivariate model including clinical information, urinary [TIMP-2]•[IGFBP7] remained statistically significant and a strong predictor of AKI (AUC 0.70, 95% CI 0.63-0.76 for clinical variables alone, versus AUC 0.86, 95% CI 0.80-0.90 for clinical variables plus [TIMP-2]•[IGFBP7]).

Conclusions. Urinary [TIMP-2]•[IGFBP7] > 0.3 $(ng/ml)^2/1000$ identifies patients at risk for imminent AKI.

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Introduction

Acute kidney injury (AKI) occurs frequently in critically ill patients, is difficult to predict and adversely impacts short and long term clinical outcomes.[1] These consequences include decreased survival;[2] increased incidence of dialysis and chronic kidney disease (CKD);[3] and markedly increased cost.[4] We recently reported the discovery and independent validation of two G1 cell cycle arrest biomarkers for AKI, tissue inhibitor of metalloproteinases (TIMP)-2 and insulin-like growth factor binding protein (IGFBP)7.[5] These markers demonstrated excellent ability to identify patients at risk of imminent (within 12 hours) AKI defined according to KDIGO (Kidney Disease: Improving Global Outcomes) criteria.[6] Risk for major adverse kidney events (MAKE) including death, dialysis or persistent renal dysfunction also increased with increasing values of the test.[5]

In order to guide the clinical use of the test for risk assessment, we selected a high-sensitivity cutoff using the results of our earlier studies.[5,7] In the current prospective multicenter study we validate the [TIMP-2]•[IGFBP7] test for identifying patients at high risk for developing AKI in the next 12 hours. Unlike our prior studies, we assembled a clinical adjudication committee (CAC) composed of three expert clinical nephrologists to determine whether the primary endpoint of moderate to severe AKI was reached for each patient. Clinical adjudication, with experts examining all available information including patient outcomes, has been the standard approach for biomarker studies for many years. For clinical diagnoses such as AKI, where tissue diagnosis is usually prohibitive, clinical adjudication is the most rigorous standard.[6,8] To our knowledge this is the first time such an approach has been used in determining the ability of a biomarker(s) to assess the risk for development of AKI.

Methods

Study Design and Participants

Topaz was a multicenter prospective study designed to validate the urinary [TIMP-2]•[IGFBP7] test to identify patients at high risk for imminent AKI. We enrolled 420 critically ill adult patients within 24 hours of admission to an intensive care unit (ICU) at 23 participating sites in the United States from May through December 2012 (Figure 1). All enrolled patients were considered critically ill due to significant respiratory or cardiovascular dysfunction (respiratory sequential organ failure assessment[9] (SOFA) score ≥ 2 or cardiac SOFA score ≥ 1). The presence of an indwelling urinary catheter was also a prerequisite for inclusion. Patients with documented moderate to severe AKI (KDIGO[6] stage 2 to 3) at the time of enrollment were excluded. Paired serum and urine samples for analysis of serum creatinine and the urinary [TIMP-2]•[IGFBP7] test were obtained immediately upon enrollment and then stored frozen until analysis. The primary end-point was the diagnosis of AKI within 12 hours of enrollment adjudicated by a CAC provided with all requested clinical data.

Procedures

Sample and data collection

Urine and serum supernatants were frozen, shipped on dry ice, stored at -70°C and thawed immediately prior to analysis (see Supplement for additional details). All relevant clinical data including patient demographics, prior health history, reason for ICU admission, serum creatinine and hourly urine output were collected from the hospital records and stored using electronic case-report forms stored in an anonymized password-protected dataset residing on servers at independent sites (Medidata Solutions, New York, NY).

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Adjudication of Acute Kidney Injury

The final diagnosis of AKI was adjudicated by an expert CAC of three independent nephrologists who were blinded to the biomarker results. The basis for the adjudication was the KDIGO consensus criteria (based on RIFLE/AKIN definitions for AKI)[6] corresponding to stages 2-3 (moderate to severe). The adjudicators were asked to determine whether AKI was present or absent (defined as "AKI" or "no AKI", respectively) within the 12 hours after enrollment using their expert judgment for each case. The CAC was provided all serum creatinine values for up to 6 months prior to enrollment and 72 hours after enrollment, all hourly urine output data available for up to 24 hours prior to enrollment and 72 hours after enrollment, daily fluid balance and use of diuretics. In addition, the date(s) of renal replacement therapy, death and ICU discharge were provided if occurring within the 72 hours after enrollment, as were age, sex, race, weight, reason for hospital and ICU admission and medical history. Additional information was provided to adjudicators if requested (see Supplement for additional details). If disagreement about the adjudication occurred, the majority determination was used although the adjudicators did not confer with each other.

Biomarker Assays

Urine samples were analyzed for TIMP-2 and IGFBP7 by technicians blinded to clinical data using a clinical immunoassay (NEPHROCHECK® Test and ASTUTE140® Meter, Astute Medical Inc, San Diego, CA; see the Supplementary Appendix) at three independent hospital laboratories (University of California at San Diego, University of Louisville, and ARUP Laboratories, Salt Lake City, UT). The ASTUTE140® Meter automatically multiplied the concentrations of the two biomarkers together and divided this product by 1000 to report a single numerical test result with units of $(ng/ml)^2/1000$ (the units for all [TIMP-2]•[IGFBP7] test and cutoff values in this report) (Supplement Table E1-E2). Urine from each subject was analyzed at each of the three testing sites, producing triplicate test values for each sample.

Serum samples were analyzed for creatinine at a central lab (Center for Esoteric Testing [CET], Burlington, NC) using the IDMS-traceable Jaffe method (Roche COBAS Modular D instrument).

Statistical Analysis

The primary objective was to validate the urinary [TIMP-2]•[IGFBP7] test for AKI risk assessment based on the test's ability to identify critically ill patients at high risk for imminent AKI occurring within 12 hours of test measurement. The primary analysis was sensitivity plus specificity > 1 for a [TIMP-2]•[IGFBP7] cutoff of 0.3, indicating that the [TIMP-2]•[IGFBP7] test is statistically informative. A target sample size of at least 400 evaluable subjects was selected for the study in order to achieve a minimum of 40 positive cases and provide greater than 95% power for the primary analysis. The predefined high-sensitivity test cutoff of 0.3 was determined using data from a previous study (Supplement Table E3). Secondary analyses included i) sensitivity and specificity at a predefined high-specificity cutoff of 2.0, and ii) examination of the relative risk for AKI in critically ill subjects stratified by either a single cutoff (0.3) or two cutoffs (0.3 and 2.0). Given the importance of sepsis as an etiology for AKI we also conducted a post-hoc subgroup analysis restricting to patients with sepsis.

Using multiple logistic regression we examined the performance of the [TIMP-2]•[IGFBP7] test with clinical covariates including enrollment serum creatinine. The multivariate model was constructed in a stepwise process. The first step was to assemble the list of clinical covariates as follows: any covariate that was reported to be associated ($p < 0.1$) with AKI by Kashani et al[5] were included; in addition, any of the susceptibilities or exposures defined in the KDIGO guideline[6] or any new covariate that was associated ($p < 0.1$) with the primary endpoint in the Topaz critically ill cohort were included. The second step was to test all of these covariates in bivariate models with the [TIMP-2]•[IGFBP7] test result. The third step was to construct the multivariate model with all covariates that had a $p < 0.1$ in the bivariate models and that were not components of the non-renal APACHE III score (to avoid collinearity with the score). Overall,

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collinearity between the variables in the multivariate model was assessed using Pearson correlation coefficients, which ranged from 0.02 to 0.22 in absolute value. Model performance was assessed with the Hosmer-Lemeshow test for goodness of fit.

Statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC) and R 3.0[10]. For all analyses, two-sided p-values less than 0.05 or one-sided p-values less than 0.025 were considered statistically significant. Categorical variables were analyzed using Fisher Exact test, chi-square test, or logistic regression. All performance statistics (area under the receiver operating characteristic curve (AUC ROC), sensitivity, specificity and relative risk) were calculated as empirical estimates. To account for the correlation between the three [TIMP-2]•[IGFBP7] test results per subject, confidence intervals (CI) were calculated using bootstrap sampling or closed-form variance equations for clustered binary data.[11]

Role of the funding source

The protocol was designed by the principal and co-principal investigators (JK, LC) in conjunction with the sponsor (Astute Medical, Inc) and met STROBE[12] and STARD[13] criteria. Data were collected by the investigators and analyzed by external statisticians (GD, JS, and MW). The first draft of the manuscript was written by the first author, with input from all other authors and the sponsor. The authors vouch for the accuracy and completeness of the data, the statistical analysis, and the fidelity of the study to the protocol. During the study, the investigators, participating institutions, and sponsors agreed to maintain data confidentiality. The protocol was approved by the Western Institutional Review Board (Olympia, Washington, USA) and institutional review board of each study site if requested. All patients or appropriate surrogates provided written informed consent.

Results

Baseline characteristics

The evaluable Topaz cohort included 408 adult critically ill patients from both surgical and medical ICUs (Figure 1). In Table 1, baseline characteristics for patients are shown separately for those developing and not developing AKI. Most of the patients presented with acute cardiovascular or respiratory dysfunction and sepsis at ICU admission (79%, 70% and 24%, respectively). A history of CKD was present in eight percent of the cohort. Additional exposures and susceptibilities for AKI, including use of radiocontrast (37%) and nephrotoxic medications (83%) preceding ICU admission were common.

Adjudication of primary endpoint

The primary endpoint was reached in 71 (17.4%) patients. The three adjudicators agreed on the diagnosis of AKI in 94% of cases. Fleiss' Kappa for multiple raters was 0.86 (95% CI 0.80-0.91, $p<0.001$), indicating excellent inter-rater reliability.

Validation of urinary [TIMP-2]•[IGFBP7] test results

Median time between ICU admission and sample collection for urinary [TIMP-2]•[IGFBP7] and serum creatinine was 15 hours (interquartile range 8-19 hours). Critically ill patients with a diagnosis of AKI within 12 hours had significantly higher median urinary [TIMP-2]•[IGFBP7] of 1.6 (0.7-2.8) compared to 0.3 (0.2-0.8) for those without AKI ($p<0.001$) (Figure 2).

Figure 3 shows the performance of a single measurement of the urinary [TIMP-2]•[IGFBP7] test to assess the risk for AKI within the subsequent 12 hours. The area under the receiver-operating-characteristic curve (AUC) was 0.82 (95% CI 0.76-0.88). This result corroborates our prior findings from the Sapphire study where an AUC of 0.80 (95% CI 0.76-0.83) was observed.[5] When compared to simultaneously measured serum creatinine (AUC 0.63, 95% CI 0.56-0.70),

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the urinary [TIMP-2]•[IGFBP7] test was superior (p<0.001) for assessing the risk of imminent AKI.

For risk assessment using the urinary [TIMP-2]•[IGFBP7] test, we applied a preselected cutoff of 0.3 as previously determined (Table E3).[7] With single testing, the sensitivity for the urinary [TIMP-2]•[IGFBP7] test at the 0.3 cutoff was 92% (95% CI 85-98) with a specificity of 46% (95% CI 41-52) and combined sensitivity plus specificity of 1.38 (95%CI 1.30-1.46, p<0.001) (Figure 3). Critically ill patients with urinary [TIMP-2]•[IGFBP7] test values > 0.3 had seven times the risk for AKI (95% CI 4-22) compared to those with a test value at or below the 0.3 cutoff (Figure 4A). Only 1 in 27 patients (3.7% absolute risk) with a test result of 0.3 or less would manifest AKI within 12 hours, whereas more than 1 in 4 (27% absolute risk) above 0.3 would do so.

The secondary analysis of the urinary [TIMP-2]•[IGFBP7] test at a preselected cutoff of 2.0 (Table E3)[7] demonstrated specificity of 95% (95% CI 93-97) and sensitivity of 37% (95% CI 26-47) (Figure 3). When patients were stratified by both cutoffs, the relative risk for AKI increased proportionally. Compared to critically ill patients with a test result below 0.3, those with urinary [TIMP-2]•[IGFBP7] test between 0.3 and 2.0 had five times the risk for AKI (95% CI 3-17) while those with test results > 2.0 had 17 times the risk for AKI (95% CI 9-54) (Figure 4B).

Addition of clinical information to biomarker test results

All covariates that were significant in the bivariate logistic regression model with [TIMP-2]•[IGFBP7] were included in the final multivariate model (Table 2). Urinary [TIMP-2]•[IGFBP7] remained a statistically significant and strong predictor of AKI when combined with the clinical model. The area under the curve of the combined urinary [TIMP-2]•[IGFBP7] test and clinical model of 0.86 (95%CI 0.80-0.90) was significantly (p<0.001) improved compared to the clinical model alone (AUC 0.70, 95%CI 0.63-0.76). In the sub-analysis of patients with sepsis urinary [TIMP-2]•[IGFBP7] test remained significant predictor of AKI (AUC 0.84, 95% 0.72-0.95).

Discussion

To our knowledge this is the first study that prospectively validates a pre-defined cutoff value of a clinical test for risk assessment of AKI where the endpoint was determined by clinical adjudication of experts blinded to the results of the test. The Topaz study prospectively validated the urinary [TIMP-2]•[IGFBP7] test's ability (at the 0.3 cutoff level) to identify critically ill patients at high risk for developing moderate to severe AKI within 12 hours. Clinical adjudication has been used in pivotal studies of biomarkers for other complex acute conditions such as acute decompensated heart failure[14] and myocardial infarction[15], but not for AKI. Importantly, all critically ill patients in our cohort were, by definition and clinical characteristics, at risk for AKI at study entry, but most of them (82.6%) did not develop AKI in the subsequent 12 hours. This observation illustrates the fact that in the presence of clinical risk factors for AKI like hypotension or hyoxemia, it is difficult to discriminate those who will imminently develop AKI from those who will not. Consequently widespread adoption of preventive interventions is unlikely in the absence of a test that reliably discerns those at increased risk from those at low risk. A similar challenge previously existed in the evaluation of patients with chest pain who are at risk for acute myocardial infarction, where only a minority of the patients with chest pain will actually have a myocardial infarction. Indeed this is why biomarkers for cardiac injury such as troponin are so useful in the evaluation of patients with chest pain. Similarly the commonly used Framingham Risk Score[16] (based on clinical characteristics) places many individuals into an intermediate-risk category for coronary artery disease (CAD)[17] where many cardiac events occur, treatment guidelines are unclear, and patients require further risk stratification.[18] Not surpsringly, while fewer than 20% of physicians report using CAD risk calculators,[19] nearly two-thirds of physicians underestimate the risk using clinical judgment alone.[20] The addition of biomarkers to clinical models has demonstrated increased accuracy for CAD risk assessment in the intermediate risk population[21] and improved their clinical utility by physicans.[22] Similarly, biomarkers are needed to help physicians further evaluate patients at risk for AKI. Amongst this

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cohort of heterogeneous critically ill patients closely resembling general ICU population in the United States,[23] the urinary [TIMP-2]•[IGFBP7] test at the 0.3 cutoff stratified risk for imminent AKI above and beyond clinical exposures and susceptibilities. Addition of the urinary [TIMP-2]•[IGFBP7] test increased the AUC of the clinical model from 0.70 to 0.86 while stratifying patients in more distinct risk categories, with a 7-fold increase in risk for patients in the high risk group compared to those in the lowest.

AKI remains one of the most common complications among hospitalized patients.[1,24] Few risk stratification models based on clinical factors exist and they are generally inadequate in accurately identifying individual patients at risk. Put simply, AKI does not hurt. Thus, the ability to identify patients at risk for imminent AKI has been elusive and represents an important unmet need.[25-27] The time window for intervention has been well demarcated for other acute conditions with high morbidity and mortality, including myocardial infarction (< 6 hours), stroke (< 3 hours) and severe trauma (< 1 hour).[28-30] Although, no such timeframe has been identified for AKI, hampered in part by the lack of precise early diagnostic tools, there is consensus amongst AKI experts that identification of a similar (12-hour) time window for intervention for severe AKI is important.[27] Large healthcare systems such as the National Health Service in the United Kingdom have systematically assessed the outcomes related to AKI, and found that delayed identification of patients with AKI contributes to worse outcomes.[31] AKI is a significant public health hazard, and the prevalence of AKI has nearly doubled over the past decade.[32,33] The associated morbidity is increasing, and AKI is becoming a significant cause of end-stage renal disease (ESRD), especially among younger patients.[33,34]

The methodological strengths of this study include complete follow up for the primary endpoint, clinicians and investigators blinded to the results of the test and an adjudicated endpoint by experts in the field. Perhaps the greatest strength is that these results are highly generalizable and validate the preselected [TIMP-2]•[IGFBP7] cutoff for a consistent assessment of relative

risk for AKI that should remain stable regardless of underlying prevalence. This is a critical step in translating new biomarkers into routine practice because it provides clinicians with actionable information based on a positive or negative test result. The urinary [TIMP-2]•[IGFBP7] test has now been shown to provide early risk stratification for imminent AKI in over 1,000 critically ill patients in two multi-center studies enrolling diverse groups of patients with the prevalence of major exposures for AKI, such as sepsis, similar to other reports in the literature.[5,35-38]

On the basis of these results, we conclude that the urinary [TIMP-2]•[IGFBP7] test can be used to identify critically ill patients at high risk for imminent AKI. This will enable early triage and risk stratification in a wide range of critically ill patients from the emergency room, acute admission areas and intensive care. Early identification of these at-risk patients should improve delivery of KDIGO-recommended interventions and enable prompt interventional studies for the treatment of AKI in the not-so-distant future.

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Figures

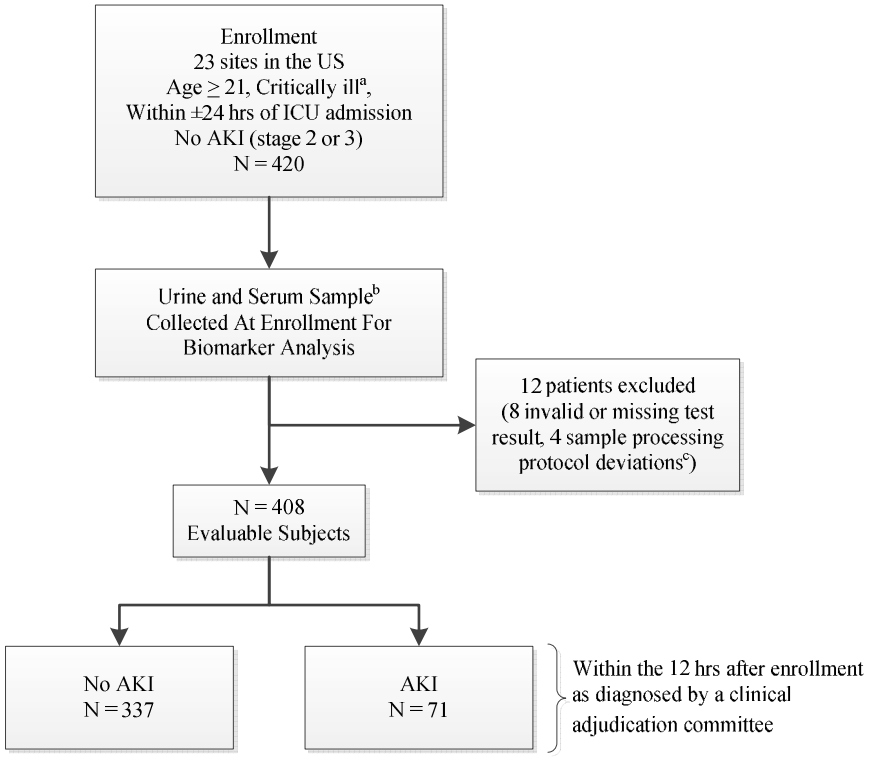

Figure 1. Design of the Topaz study of critically ill subjects. [a]Critical illness was defined as admission to an ICU and sepsis-related organ failure assessment score[9] ≥2 for respiratory and/or ≥1 for cardiovascular system. [b]Urine and Serum samples were stored at ≤-70°C until analysis. Subjects had to have an enrollment urine sample (but not a serum sample) to be considered evaluable (405 subjects had a study-specific enrollment serum sample). [c]Criteria for excluding samples were predefined prior to analysis.

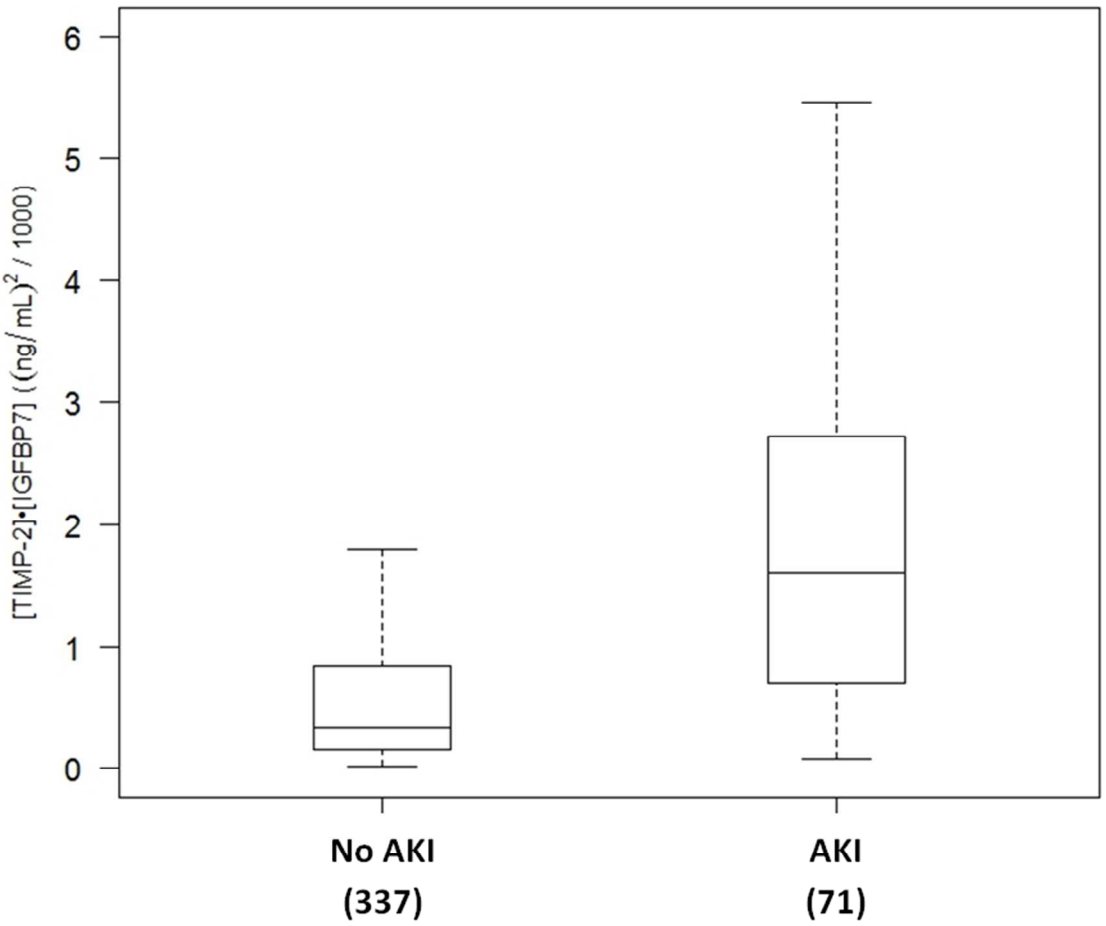

Figure 2. Box plots of urinary [TIMP-2]•[IGFBP7] test values. Boxes and whiskers show inter-quartile ranges and total observed ranges (censored at 1.5 times the inter-quartile ranges), respectively. Subjects with AKI had significantly higher [TIMP-2]•[IGFBP7] test values than subjects without AKI (Wilcoxon rank sum test $p<0.001$).

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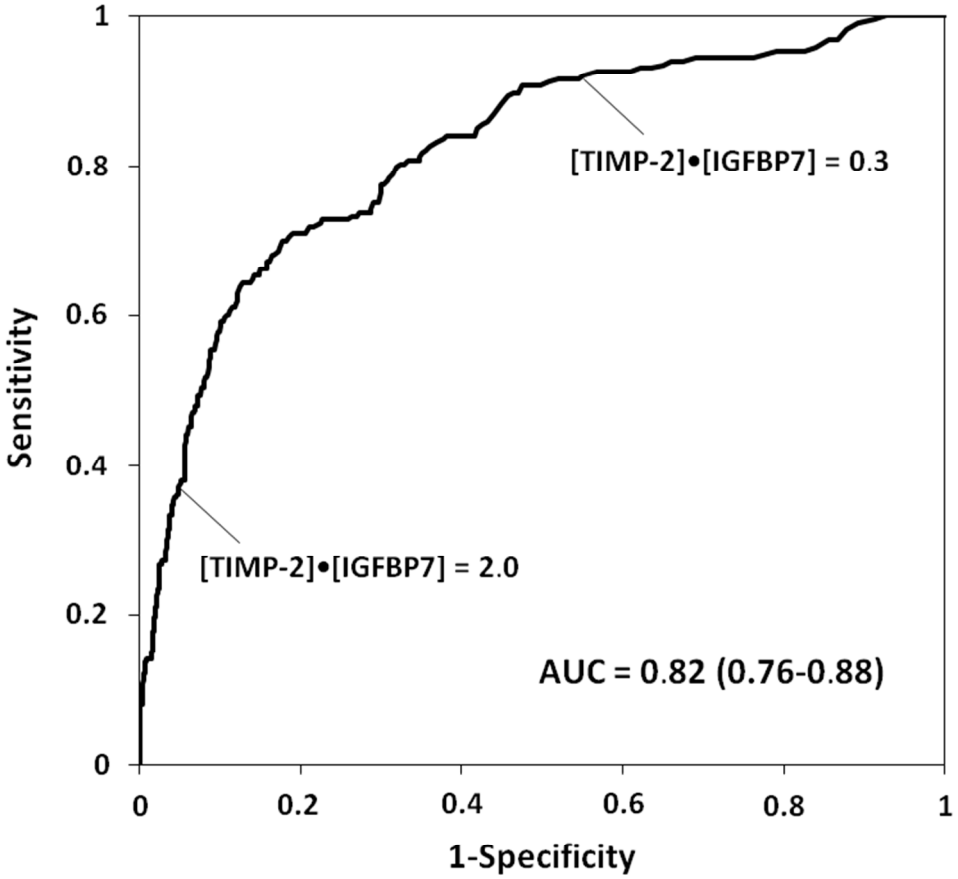

Cutoff value, $(ng/ml)^2/1000$	Sensitivity, % (95% CI)	Specificity, % (95% CI)
0.3	92 (85-98)	46 (41-52)
2.0	37 (26-47)	95 (93-97)

Figure 3. Urinary [TIMP-2]•[IGFBP7] test ROC curve for discriminating critically ill subjects with AKI (N = 71) from those with no AKI (N =337).

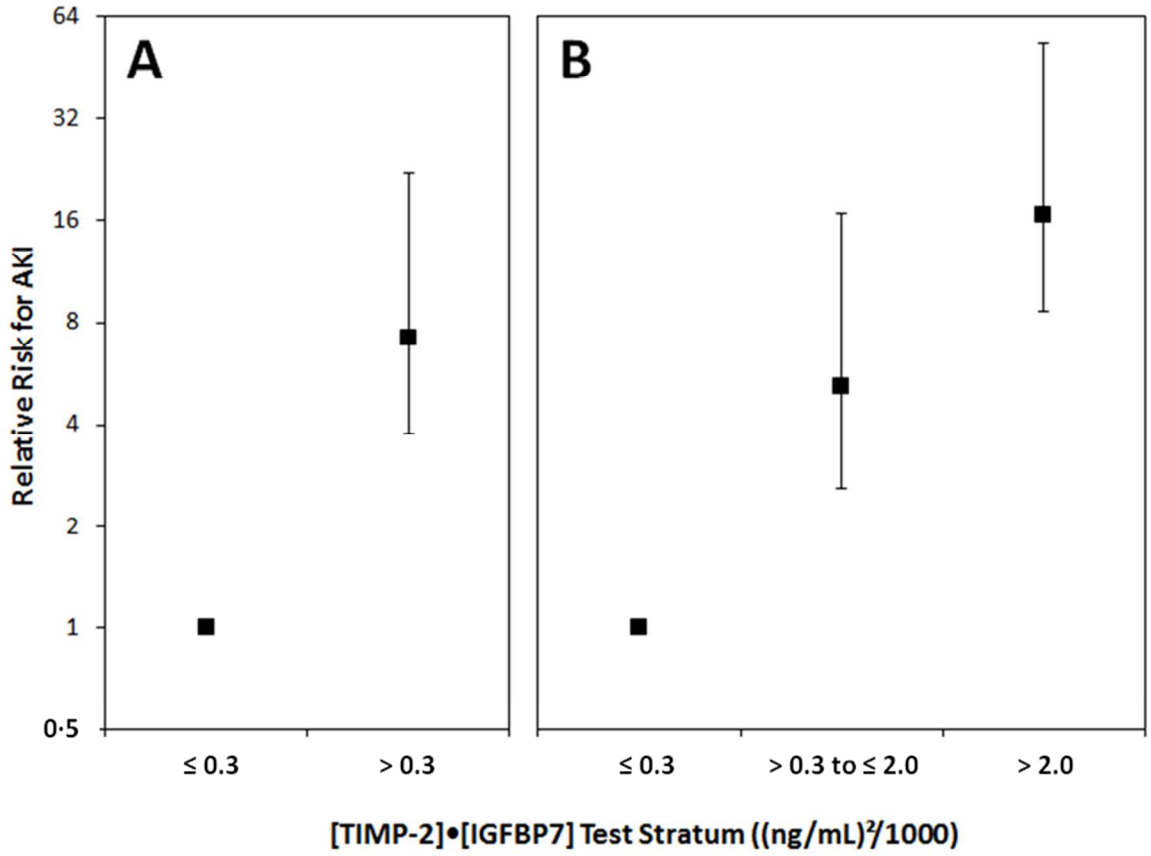

Figure 4. Relative risk of AKI in [TIMP-2]•[IGFBP7] test strata. AKI risk (A) in stratum with [TIMP-2]•[IGFBP7] values > 0.3 relative to risk in stratum ≤ 0.3, and (B) in strata with [TIMP-2]•[IGFBP7] values (i) between 0.3 and 2.0 and (ii) > 2.0, relative to risk in stratum ≤ 0.3. P-values < 0.002 for all pairwise strata comparisons. Error bars are 95% CI. 71 AKI subjects, 337 no AKI. We observed an absolute risk in the ≤ 0.3 stratum of 3.7%; whereas above the 0.3 cuttoff absolute risk was 27%. When two cutoffs are used absolute risk for the >0.3 ≤ 2.0 stratum was 19% while above 2.0 absolute risk was 62%.

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Table 1. Baseline characteristics

	No AKI	AKI	All patients
Patients, n (%)	337 (83%)	71 (17%)	408
Age, years*	63 (17)	62 (16)	63 (17)
Male	184 (55%)	35 (49%)	219 (54%)
Race			
Black	47 (14%)	9 (13%)	56 (14%)
White	280 (83%)	59 (83%)	339 (83%)
Other/Unknown	10 (3%)	3 (4%)	13 (3%)
ICU Type			
Cardiac Medical/Surgical	40 (12%)	8 (11%)	48 (12%)
Medical	149 (44%)	31 (44%)	180 (44%)
Mixed/Other	69 (20%)	14 (20%)	83 (20%)
Surgical/Trauma	79 (23%)	18 (25%)	97 (24%)
Medical History			
Body Mass Index, kg/m^2†	28 (24-34)	31 (26-38)	28 (25-34)
Chronic Kidney Disease	27 (8%)	5 (7%)	32 (8%)
Diabetes Mellitus	92 (27%)	24 (34%)	116 (28%)
Congestive Heart Failure	67 (20%)	18 (25%)	85 (21%)
Coronary Artery Disease	100 (30%)	21 (30%)	121 (30%)
Hypertension	221 (66%)	50 (70%)	271 (66%)
Chronic Obstructive Pulmonary Disease	80 (24%)	10 (14%)	90 (22%)
Cancer	90 (27%)	19 (27%)	109 (27%)
Hematocrit < 30%	159 (47%)	25 (35%)	184 (45%)
Liver Disease	16 (5%)	8 (11%)	24 (6%)
Acute Exposures and Susceptibilities			
APACHE III Score (non-renal)†, ‡	56 (44-75)	66 (49-87)	58 (44-78)
Cardiovascular dysfunction§	261 (77%)	60 (85%)	321 (79%)
Respiratory dysfunction¶	231 (69%)	53 (75%)	284 (70%)
Sepsis‖	75 (22%)	22 (31%)	97 (24%)
Radiocontrast agents	123 (36%)	28 (39%)	151 (37%)
Nephrotoxic drugs (at least 1)**	276 (82%)	61 (86%)	337 (83%)
Nephrotoxic drugs (at least 2)**	175 (52%)	32 (45%)	207 (51%)
Enrollment Serum Creatinine, mg/dL†	0.9 (0.7-1.2)	1.1 (0.8-1.5)	0.9 (0.7-1.3)

*Mean (standard deviation); †median (interquartile range); ‡excludes serum creatinine, urine output and blood urea nitrogen; §cardiovascular SOFA ≥ 1; ¶respiratory SOFA ≥ 2; ‖reason for ICU admission; **includes any of the following medications administered within 5 days prior to enrollment: nonsteroidal anti-inflammatory drugs, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, immunosuppressants, beta lactam antibiotics, aminoglycosides, vancomycin, acyclovir, amphotericin, allopurinol, colistin.

Table 2. Multivariate logistic regression model of clinical risk factors and the urinary [TIMP-2]•[IGFBP7] test[a]

Variable[b]	Adjusted Odds Ratio (95% CI)	
	Without Urinary [TIMP-2]•[IGFBP7]	With Urinary [TIMP-2]•[IGFBP7]
Enrollment Serum Creatinine[c] (per unit log)	2.36 (0.74-7.50)	2.45 (0.68-8.84)
APACHE III Score (non-renal) (per unit)	1.02 (1.01-1.03)***	1.02 (1.01-1.03)**
Body Mass Index (per kg/m^2)	1.05 (1.02-1.08)***	1.07 (1.04-1.11)***
Urinary [TIMP-2]•[IGFBP7] test[c] (per unit log)	Not included	16.5 (7.6-35.5)***
Area under the curve (95% CI)	0.70 (0.63-0.76)***	0.86 (0.80-0.90)***

[a]The endpoint was a final diagnosis of AKI as shown in Figure 1; only the subjects who had a value recorded for all 4 variables in the model were included in the multivariate analysis (N = 324 No AKI, 69 AKI, 393 total). [b]The three AKI risk factors included in the multivariate model where those with $p < 0.1$ in bivariate logistic regression with the [TIMP-2]•[IGFBP7] test (performed for 17 risk factors including chronic kidney disease, sepsis and use of nephrotoxic agents, details presented in the Supplement). [c]Log_{10} transformation of the values measured in the enrollment samples were used in the model.**p<0.01, ***p<0.001. p = 0.45 and 0.36 for the Hosmer-Lemeshow goodness of fit test for the model without and with [TIMP-2]•[IGFBP7], respectively, which indicates good fit.

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Supplementary Appendix

Validation Of Cell-Cycle Arrest Biomarkers For Acute Kidney Injury Using Clinical Adjudication

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Contents

Topaz Study Sites and Personnel

Topaz Principal Investigator: John A Kellum, MD

Topaz Co-Principal Investigator: Lakhmir S Chawla, MD

Topaz Study Enrolling Sites:

California: *Loma Linda University* – H Bryant Nguyen, MD; Michael Terry, RCP; Jaenette Merrill-Henry, RCP; Deborah Dodge, RCP. *University of California at Los Angeles* – Eric Kleerup, MD.

District of Columbia: *George Washington University Medical Center* – Danielle Davison, MD; Linda Shehu; Ermira Mitchell; Christina Seneff.

Florida: *Tampa General Hospital* – Jason Wilson, MD. *University of Florida* – Azra Bihorac, MD; Philip Efron, MD; Adrian Agudelo; Hassan Alnuaimat, MD.

Idaho: *Eastern Idaho Medical Consultants, LLC* – Kenneth Krell, MD; Amy Thornley, ACNP-BC; Kevin B Foster, CPhT.

Illinois: *Northwestern University* – Richard Wunderink, MD; Helen Donnelly; Margaret Travis. *University of Chicago* – Jay Koyner, MD; Sharon Trevino, RN.

Louisiana: *Louisiana State University* – Derrel D Graham, MD; Kimberley Hutchinson, RN.

Maryland: *University of Maryland School of Medicine* – Matthew Lissauer, MD; Jessica Warren; Holly Howes.

Michigan: *University of Michigan* – Michael Heung, MD; Anthony Courey, MD; Theresa Mottes, RN; Michigan Clinical Research Unit.

Minnesota: *Hennepin County Medical Center* – James Miner, MD.

Montana: *The International Heart Institute of Montana* – Richard Sellman, MD.

North Carolina: *Durham VA Medical Center* – Andrew D Shaw, MD; S Becky Perfect; Elizabeth Rogers.

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Ohio: *Summa Health System Akron City Hospital* – Scott T Wilber, MD; Bradley R Martin, MD; James A Wilson, MD; Jennifer A Frey, PhD, CCRP.

Oregan: *Portland VA Medical Center* – Jennifer LeTourneau, DO; Kathy A Avalos, MA.

Pennsylvania: *AGH Nephrology Associates* – Luis Ortega, MD; Donna Creese; Khaled Nashar, MD. *University of Pittsburgh Medical Center* – Ali Al-Khafaji, MD; Denise Scholl; Barb Early; Sarah Cua.

Tennessee: *Vanderbilt University School of Medicine* – Wesley H Self, MD; Dayna Wyatt, RN; Charity Graves.

Texas: *Houston Methodist Medical Center* – Janice L Zimmerman, MD; Deepa Bangalore, MD; Raul Sanchez Leon, MD.

Virginia: *Virginia Commonwealth University* – Kyle Gunnerson, MD; Tamara Ponton, RN; Jennifer Chadbourne, RN.

Topaz Study Measuring Sites:

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Kentucky: *University of Louisville* – Saeed Jortani, PhD; William W Tucker; Louise Isaacs; Gina Thomson; Sanda Jones.

Utah: *ARUP Laboratories* – Joely Straseski, PhD.

Supplementary Methods

Topaz Study

Critically ill subjects in the Topaz study were enrolled at 23 clinical sites in the United States (see Figure 1 in the manuscript). Baseline and demographic information is shown in Table 1 of the manuscript. Entry and evaluability criteria were as follows:

Inclusion Criteria

a. Males and females 21 years of age or older;

b. Subjects must be enrolled (first study-specific sample collection) within 24 hours of ICU admission;

- Subjects enrolled from ED or Floor must be admitted to the ICU within 24 hours of enrollment;
- Subjects enrolled in the ICU must have been admitted to the ICU or transferred into the study ICU from another ICU no more than 24 hours prior to enrollment;

c. Expected to remain in the ICU for at least 48 hours after enrollment;

d. Use of indwelling urinary catheter as standard care expected for at least 48 hours after enrollment;

e. At least one of the following acute conditions documented within 24 hours prior to enrollment:

- Respiratory SOFA score of ≥ 2 ($PaO_2/FiO_2 < 300$);
- Cardiovascular SOFA score of ≥ 1 (MAP < 70 mm Hg and/or any vasopressor required);

f. Subject (or authorized representative) able and willing to provide written informed consent for study participation.

Exclusion Criteria

a. Special populations including women with known pregnancy, prisoners or institutionalized individuals;

b. Previous renal transplantation;

c. Known moderate to severe AKI prior to enrollment (e.g., RIFLE-I or RIFLE-F/ AKIN 2 or AKIN);

d. Already receiving dialysis (either acute or chronic) or in imminent need of dialysis at the time of enrollment;

e. Known infection with human immunodeficiency virus (HIV) or active hepatitis (acute or chronic);

f. Subjects with a history of Chronic Kidney Disease (CKD) without a baseline serum creatinine value (baseline within 6 months of enrollment).

Evaluability Criteria

Subjects will be considered enrolled in the study when the enrollment (first) study–specific blood and urine samples are obtained. Subjects who terminate participation prior to that point will not be considered formally enrolled and may be replaced.

A subject will be considered unevaluable for the primary purposes of this study if he/she does not have an indwelling urinary catheter and urine output data obtained through 30 hours after enrollment. For each draw time (enrollment and within 10-18 hours of enrollment) urine and serum samples collected more than 1 hour apart during a draw time will be considered unevaluable. Subjects discharged from the ICU prior to 30 hours in the study will be considered unevaluable. Subjects who expire prior to 48 hours in the study will be considered evaluable as long as he/she has study blood / urine samples obtained at enrollment and urine output data available through time of death. Subjects who were not already receiving kidney-related dialysis (either acute or chronic) or in imminent need of

dialysis at the time of enrollment, but who received kidney-related dialysis within 48 hours of enrollment will be considered evaluable. Subjects receiving non-kidney related dialysis or ultrafiltration within 12 hours of the first sample collection will be considered unevaluable; if non-kidney dialysis or ultrafiltration is initiated within 12 hours of collection of a second sample, that second sample will be considered non-evaluable. Subjects who are unevaluable will be withdrawn from the study.

Laboratory Methods

Blood and Urine Samples

Study-specific blood samples were obtained via direct venipuncture, via other available venous access (e.g., an existing femoral sheath, central venous line, peripheral intravenous line, or hep-lock) or via an indwelling arterial line. Blood was collected in clot activator blood collection tubes (for serum). Serum was prepared by standard methods (centrifugation for 10 minutes at a minimum of 1,300 x g after clotting) and aliquots were frozen (on dry ice or liquid nitrogen) within two hours of collection. Samples were shipped on dry ice and stored at ≤ -70° C. Samples were thawed immediately prior to measurement. A blood sample was considered unevaluable for the study if:

- It contained an insufficient volume (tube contains less than half of fill volume);
- It was not collected in the proper type of blood collection tube;
- The specimen was hemolyzed (i.e. a serum creatinine value cannot be obtained);
- It was not properly labeled with a barcode label;
- It was not collected within 60 minutes of the corresponding urine sample;
- It was not processed within 120 minutes (2 hours) of sample collection;
- It was not properly frozen at the site or upon arrival after shipping.

Study-specific urine samples were collected in standard (non-coated) specimen cups. For subjects with indwelling bladder catheters, the collection bag was first emptied and then a fresh sample of urine collected; alternatively the sample could be taken from a urometer, if present. Samples were centrifuged (10 minutes at 1,000 x g) to remove any cells or other debris, and aliquots were frozen (on dry ice or liquid nitrogen) within 2 hours of collection. Samples were shipped on dry ice and stored at ≤ -70° C. Samples were thawed immediately prior to measurement. A urine sample was considered unevaluable if:

- It was not collected within 60 minutes of the corresponding blood sample;
- It was not processed and frozen within 120 minutes (2 hours) of sample collection;
- There was less than 15 mL of sample;
- It was not properly labeled with a barcode label;
- It was not properly frozen upon arrival after shipping.

After collection and processing, study-specific serum and urine samples were shipped to the study sponsor, who banked them and shipped them to the laboratories where they were analyzed.

Assay Methods

The biomarkers TIMP-2 and IGFBP7 were measured with the NEPHROCHECK® Test (Astute Medical, San Diego, CA) at three independent hospital laboratories. The test is an *in vitro* diagnostic device that quantitatively measures IGFBP7 and TIMP-2 in human urine by fluorescence immunoassay on the ASTUTE140® Meter. The test is a single-use cartridge comprised of assays for the two biomarkers on a membrane test strip enclosed in a plastic housing that employs a sandwich immunoassay technique. The test procedure involves the operator applying a fresh or thawed (i.e. previously frozen) clinical urine sample (mixed with labeled fluorescent conjugate) to the test cartridge, and

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then inserting the test cartridge into the meter for incubation, reading, result calculation, and result display in about 20 minutes. The meter is a bench-top/table-top analyzer that converts the fluorescent signal from each of the two immunoassays into concentrations (of TIMP-2 and IGFBP-7) and computes and displays a single numerical test result equal to the product of the concentrations ([TIMP-2]•[IGFBP7]) in units of $(ng/ml)^2/1000$. Concentration results for the assays are traceable to reference standard solutions that contain defined mass (concentration) of TIMP-2 and IGFBP-7 in accordance with EN ISO 17511.[E1] Each test cartridge contains two detection zones used as internal controls (one positive and one negative control). These positive and negative controls are run automatically with every sample, in order to confirm the integrity of the test cartridge and the performance of the meter. If the automatic check of these internal controls shows that the control value results are not within pre-defined limits, the meter will display an error message and the test result will not be reported. These controls are in addition to external liquid controls (traceable to the same reference standard solutions as the test) which are run to verify test performance and operator proficiency. Data on the analytical characteristics of the test were obtained from the manufacturer are shown in Tables E1 and E2. The test and meter were not available for commercial sale in the United States at the time of manuscript submission.

Enrollment serum samples were analyzed for serum creatinine at a central lab (Center for Esoteric Testing [CET], Burlington, NC) using the Jaffe method (Roche COBAS Modular D instrument) with IDMS-traceable calibration.

Clinical Adjudication

We recruited three noted experts in clinical nephrology to serve as adjudicators:

Kathleen D. Liu, MD, PhD, MAS
Associate Professor, Division of Nephrology and Critical Care
Departments of Medicine and Anesthesia
University of California, San Francisco

Mitchell H. Rosner, MD, FACP
Professor of Medicine, Division of Nephrology
University of Virginia Health System

Anitha Vijayan, MD
Professor of Medicine, Renal Division
Washington University School of Medicine in St. Louis

These experts were independent of the sponsor and neither they nor their institutions participated in the sample collection or sample measurement studies. Adjudicators were paid for their work but had no other relationship with the sponsor.

The procedures for adjudication were defined in advance of the study by consensus amongst the three adjudicators and the principal investigator in a series of face-to-face and teleconference meetings. Adjudicators collectively determined which variables would be extracted from the medical record and provided to them for adjudication. These variables included all serum creatinine values for up to 6 months prior to enrollment and 72 hours after enrollment, all hourly urine output data available for up to 24 hours prior to enrollment and 72 hours after enrollment, daily fluid balance and use of diuretics. In addition, the date(s) of renal replacement therapy, death and ICU discharge were provided if occurring within the 72 hours after enrollment, as were age, sex, race, weight, reason for hospital and ICU admission and medical history. Adjudicators could also request additional information for individual patients. The procedures and the precise clinical variables used were finalized prior to the start of enrollment and adjudication was completed prior to sample testing and analysis.

Each adjudicator was provided a form for each patient containing all the clinical information described above and identified only by anonymized identification number. Adjudicators indicated their diagnosis ("AKI" or "No AKI") independently without consultation with each other. They had no knowledge of biomarker results at any time. The forms were provided to the external statisticians for analysis.

The diagnosis of AKI was determined by each adjudicator. The basis for the adjudication was the KDIGO consensus criteria (based on RIFLE/AKIN definitions for AKI)[E2] corresponding to stages 2-3 (moderate to severe). The

adjudicators were asked to determine whether AKI was present or absent (defined as "AKI" or "no AKI", respectively) within the 12 hours after enrollment and sample collection based on these criteria, but were free to use their expert judgment for each case. For example, an adjudicator could diagnose AKI in a patient dying prior to reaching KDIGO criteria. A two thirds majority was predefined for use as the final adjudication.

Statistical Analysis

General analysis: Statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC) and R 3.0.[E3] For all analyses, two-sided p-values less than 0.05 or one-sided p-values less than 0.025 were considered statistically significant. Categorical variables were analyzed using Fisher Exact test, chi-square test, or logistic regression. All performance statistics (area under the ROC curve (AUC), sensitivity, specificity, NPV, PPV, likelihood ratios, and relative risk) were calculated as empirical estimates. Logistic regression analyses used the log transform the serum creatinine and [TIMP-2]•[IGFBP7] test results.

Treatment of multiple test results: Each urine sample was measured with the [TIMP-2]•[IGFBP7] test at three independent hospital laboratories, resulting in three test values for every subject. For the analysis presented in Figures 3 and 4 and Table E4, all three test results were included in a clustered analysis. Confidence intervals were calculated using closed-form variance equations for clustered binary data for sensitivity, specificity, NPV and PPV and using bootstrap sampling for all other analyses.[E4] For the analysis presented in Figure 2 and Table 2, the median of the three test results was used.

Multivariate model: The multivariate model shown in Table 2 with clinical risk factors for AKI and the [TIMP-2]•[IGFBP7] test was constructed in a stepwise process. The first step was to assemble a list of clinical covariates that were associated with AKI. This list was assembled as follows: any covariate that was reported to be associated ($p < 0.1$) with AKI by Kashani et al[E5] (shown in table E4 in Kashani et al) were included; in addition, any of the susceptibilities or exposures defined in the KDIGO guideline[E2] (Table 6 in the KDIGO Guideline) or any covariate from Table 1 of the manuscript that was associated ($p < 0.1$) with the AKI endpoint in the Topaz critically ill cohort were included. The second step was to test all of these covariates (Age, BMI, enrollment serum creatinine, APACHE excluding renal function assessment, Sex, Race, history of hypertension, use of nephrotoxic drugs, chronic liver disease, diabetes mellitus, history of chronic kidney disease, chronic obstructive pulmonary disease, anemia on ICU admission, congestive heart failure, sepsis on ICU admission, major surgery prior to ICU admission, cerebrovascular disease on ICU admission) in bivariate models with the [TIMP-2]•[IGFBP7] test result. The third step was to construct the multivariate model (Tables 2) with all covariates that had $p < 0.1$ in the bivariate models and that were not components of the non-renal APACHE III score. Three covariates (serum creatinine, body mass index and non-renal APACHE III score) met this criterion for inclusion in the multivariate model.

Supplementary Results

Tables E1 and E2 show analytical characteristics of the clinical assay used to measure TIMP-2 and IGFBP7. Table E3 shows a sensitivity analysis of operating characteristics for the [TIMP-2]•[IGFBP7] test over a range of possible cutoff values from 0.1 to 2.5 $(ng/ml)^2/1000$ for the previously reported Sapphire cohort[E5] and are the data used to predefine the high-sensitivity test cutoff at 0.3 and the high-specificity test cutoff at 2.0 (defined and locked prior to measurement of the Topaz validation study samples). Table E4 shows a sensitivity analysis of operating characteristics for the Topaz validation study at various cutoff values.

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Tables

Table E1. Analytical sensitivity and reportable range of the clinical assay for the Urinary [TIMP-2] • [IGFBP7] test (NephroCheck® Test).

Biomarker	Limit of Blank	Limit of Detection	Limit of Quantitation	Reportable Range
TIMP-2	0.5 ng/mL	0.7 ng/mL	0.7 ng/mL	1.2–225 ng/mL
IGFBP7	0.4 ng/mL	2.8 ng/mL	2.8 ng/mL	20–600 ng/mL
[TIMP-2]•[IGFBP7]/1000	0.0002	0.002	0.002	0.02–135

The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) values for each biomarker assay contained in the NEPHROCHECK® Test were determined in accordance with the methods provided in CLSI guideline EP17-A[E6], from measurements conducted with three different lots of test devices. The linearity of the biomarker assays contained in the NEPHROCHECK® Test was evaluated in accordance with the methods provided in CLSI guideline EP6-A[E7] with three different lots of test devices. Assay results were found to be linear between 0.7 and 284.1 ng/mL for the TIMP-2 assay and 1.0 and 649.8 ng/mL for the IGFBP7 assay, i.e., over the entire reportable range.

Table E2. Reproducibility of the clinical assay for the urinary [TIMP-2]•[IGFBP7] test (NephroCheck® Test)

	Biomarker Concentration			Total Imprecision (%CV)		
Sample	**IGFBP7 (ng/mL)**	**TIMP-2 (ng/mL)**	**[TIMP-2] • [IGFBP7] (ng/mL)^2/1000**	**IGFBP7 (ng/mL)**	**TIMP-2 (ng/mL)**	**[TIMP-2] • [IGFBP7] (ng/mL)^2/1000**
Low	56	2.5	0.14	6.8%	9.3%	13.7%
Medium	262	122	32	6.2%	4.8%	10.4%
High	519	195	102	6.1%	5.2%	10.7%

The reproducibility of the concentration results of each individual biomarker, as well as the [TIMP-2]•[IGFBP7] test result were determined in accordance with the methods provided in CLSI guideline EP5-A2[E8]. The reproducibility of results was determined at three testing sites under intended use conditions using three NEPHROCHECK® Test Kit lots. At each site, three urine test samples containing low, medium and high analyte concentrations that spanned the measurable range of the test were tested with at least 80 replicates. These data were collected over at least 40 separate runs that were conducted twice a day over at least 20 total days of testing. The Total Imprecision represents the total test variability including within-day, between-day, between-operator and between-device lot variability.

Table E3. Operating characteristics (95% CI) as a function of [TIMP-2]•[IGFBP7] test cutoff value for discriminating subjects with AKI from subjects with no AKI in the Sapphire cohort of critically ill subjects.

Cut-off ((ng/mL)^2/1000)	Sensitivity[a], %	Specificity[a], %	Negative Likelihood Ratio[a]	Positive Likelihood Ratio[a]	Negative Predictive Value[b], %	Positive Predictive Value[b], %
0.1	97 (94-100)	22 (20-25)	0.12 (0.00-0.27)	1.25 (1.20- 1.30)	99 (97-100)	14 (13-15)
0.2	93 (88-97)	38 (35-41)	0.19 (0.07-0.33)	1.50 (1.39- 1.61)	98 (96-99)	16 (15-17)
0.3	89 (82-94)	50 (47-53)	0.22 (0.11-0.35)	1.79 (1.63- 1.97)	97 (96-99)	18 (17-20)
0.4	80 (72-87)	58 (54-61)	0.35 (0.23-0.49)	1.89 (1.66- 2.15)	96 (94-97)	19 (17-21)
0.5	74 (66-82)	65 (62-68)	0.40 (0.27-0.53)	2.12 (1.82- 2.45)	95 (94-97)	21 (18-24)
0.6	71 (63-80)	71 (68-74)	0.40 (0.28-0.53)	2.50 (2.10- 2.91)	95 (94-97)	24 (21-27)
0.7	70 (61-79)	76 (73-79)	0.39 (0.28-0.51)	2.92 (2.44- 3.44)	95 (94-97)	27 (23-31)
0.8	65 (56-74)	80 (77-82)	0.44 (0.32-0.56)	3.17 (2.62- 3.80)	95 (93-96)	29 (25-33)
0.9	62 (52-71)	82 (79-85)	0.47 (0.36-0.58)	3.43 (2.78- 4.15)	94 (93-96)	30 (26-35)
1.0	59 (49-69)	84 (82-86)	0.49 (0.37-0.60)	3.67 (2.92- 4.52)	94 (93-95)	32 (27-37)
1.1	57 (47-67)	86 (83-88)	0.50 (0.38-0.62)	3.97 (3.13- 5.04)	94 (93-95)	33 (28-39)
1.2	54 (45-64)	87 (85-89)	0.53 (0.42-0.64)	4.17 (3.24- 5.36)	94 (92-95)	34 (29-40)
1.3	51 (41-60)	88 (86-90)	0.56 (0.45-0.66)	4.39 (3.38- 5.71)	93 (92-95)	36 (30-42)
1.4	46 (37-56)	90 (88-92)	0.60 (0.49-0.71)	4.54 (3.43- 6.05)	93 (92-94)	36 (30-43)
1.5	46 (37-56)	91 (89-93)	0.59 (0.49-0.70)	4.99 (3.73- 6.66)	93 (92-94)	39 (32-46)
1.6	45 (36-55)	92 (90-93)	0.60 (0.49-0.69)	5.39 (4.01- 7.27)	93 (92-94)	40 (34-48)
1.7	45 (36-54)	93 (91-94)	0.60 (0.50-0.69)	6.08 (4.56- 8.41)	93 (92-94)	43 (36-51)
1.8	44 (34-53)	94 (92-95)	0.60 (0.50-0.70)	6.82 (5.00- 9.48)	93 (92-94)	46 (38-55)
1.9	42 (33-52)	94 (93-96)	0.61 (0.51-0.71)	7.16 (5.19-10.09)	93 (92-94)	47 (39-56)
2.0	42 (33-51)	95 (93-96)	0.62 (0.51-0.71)	7.69 (5.57-10.88)	93 (92-94)	49 (41-58)
2.1	41 (32-51)	95 (94-96)	0.62 (0.52-0.72)	8.33 (5.96-11.84)	93 (92-94)	51 (43-60)
2.2	40 (31-50)	96 (94-97)	0.63 (0.53-0.73)	8.96 (6.25-13.08)	93 (91-94)	53 (44-62)
2.3	38 (29-48)	96 (95-97)	0.64 (0.54-0.74)	9.75 (6.74-14.02)	93 (91-94)	55 (46-64)
2.4	36 (27-46)	96 (95-97)	0.66 (0.56-0.76)	9.66 (6.43-13.98)	92 (91-93)	55 (45-64)
2..5	34 (26-44)	96 (95-97)	0.68 (0.59-0.77)	9.56 (6.29-14.12)	92 (91-93)	55 (44-64)

The operating characteristics show the performance of the [TIMP-2]•[IGFBP7] test for discriminating between Sapphire subjects with and without KDIGO Stage 2-3 AKI within 12 hours of sample collection.[5] These results were used to prospectively select the cutoffs validated in the Topaz study, prior to measurement of the Topaz samples.
[a]Sensitivity, specificity and likelihood ratios are operating characteristics that are independent of prevalence of the endpoint (AKI), and therefore provide the most consistent estimation of test performance that can be expected across different patient cohorts that may have different prevalence of AKI. [b]Negative and positive predictive values depend on the prevalence of the endpoint (AKI) and can be calculated from the test sensitivity and specificity and the prevalence. These numbers should be adjusted for the expected prevalence if applied to populations with substantially different prevalence.

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Table E4. Operating characteristics (95% CI) as a function of [TIMP-2]•[IGFBP7] test cutoff value for discriminating subjects with AKI from subjects with no AKI in the Topaz cohort of critically ill subjects.

Cutoff ((ng/ml)^2/1000)	Sensitivity[a], %	Specificity[a], %	Negative Likelihood Ratio[a]	Positive Likelihood Ratio[a]	Negative Predictive Value[b], %	Positive Predictive Value[b], %
0.1	97 (93-100)	14 (11-18)	0.23 (0.03-0.53)	1.1 (1.1-1.2)	95 (91-100)	19 (15-23)
0.2	94 (88-99)	34 (29-39)	0.18 (0.04-0.36)	1.4 (1.3-1.6)	96 (93-100)	23 (18-28)
0.3	92 (85-98)	46 (41-52)	0.18 (0.06-0.33)	1.7 (1.5-1.9)	96 (93-99)	27 (21-32)
0.4	88 (81-95)	55 (50-60)	0.21 (0.09-0.36)	2.0 (1.7-2.3)	96 (93-98)	29 (23-35)
0.5	84 (75-92)	62 (57-67)	0.26 (0.14-0.41)	2.2 (1.9-2.6)	95 (92-98)	32 (25-38)
0.6	80 (71-89)	67 (62-72)	0.29 (0.17-0.43)	2.4 (2.0-2.9)	94 (91-97)	34 (27-41)
0.7	75 (65-85)	70 (66-75)	0.35 (0.22-0.50)	2.5 (2.1-3.1)	93 (90-96)	35 (27-42)
0.8	73 (62-83)	74 (70-79)	0.37 (0.23-0.52)	2.8 (2.3-3.5)	93 (90-96)	37 (29-45)
0.9	72 (62-83)	78 (73-82)	0.36 (0.22-0.50)	3.2 (2.5-4.1)	93 (90-96)	41 (32-49)
1.0	70 (59-80)	82 (78-86)	0.37 (0.24-0.50)	3.9 (3.0-5.1)	93 (90-96)	45 (36-54)
1.1	66 (55-77)	85 (81-88)	0.40 (0.27-0.53)	4.3 (3.2-5.8)	92 (89-95)	48 (38-57)
1.2	64 (53-75)	86 (83-90)	0.41 (0.29-0.55)	4.7 (3.4-6.5)	92 (89-95)	50 (40-60)
1.3	61 (50-72)	88 (85-92)	0.44 (0.32-0.57)	5.3 (3.9-7.6)	91 (88-94)	53 (42-63)
1.4	58 (47-69)	90 (87-93)	0.47 (0.35-0.59)	5.9 (4.2-8.7)	91 (88-94)	55 (44-66)
1.5	54 (43-65)	91 (88-94)	0.50 (0.38-0.63)	6.3 (4.3-9.6)	90 (87-93)	57 (45-68)
1.6	50 (39-61)	92 (90-95)	0.54 (0.42-0.66)	6.6 (4.4-10.2)	90 (87-93)	58 (46-70)
1.7	47 (36-59)	93 (91-96)	0.56 (0.44-0.69)	6.9 (4.5-11.5)	89 (86-93)	59 (47-72)
1.8	44 (33-55)	94 (92-96)	0.59 (0.47-0.71)	7.4 (4.7-12.7)	89 (86-92)	61 (48-74)
1.9	40 (29-51)	94 (92-97)	0.64 (0.52-0.75)	7.1 (4.4-12.4)	88 (85-91)	60 (46-73)
2.0	37 (26-47)	95 (93-97)	0.67 (0.55-0.78)	7.7 (4.5-14.1)	88 (84-91)	62 (48-76)
2.1	35 (24-45)	96 (94-98)	0.68 (0.56-0.79)	8.3 (4.9-16.2)	87 (84-91)	64 (49-78)
2.2	33 (23-44)	96 (94-98)	0.69 (0.58-0.80)	8.8 (5.0-18.0)	87 (84-91)	65 (50-80)
2.3	31 (21-42)	97 (95-98)	0.71 (0.60-0.81)	9.1 (5.0-18.9)	87 (84-90)	66 (50-81)
2.4	29 (19-39)	97 (95-99)	0.73 (0.63-0.84)	8.6 (4.6-19.1)	87 (83-90)	65 (48-81)
2.5	28 (18-38)	97 (95-99)	0.75 (0.64-0.85)	8.7 (4.6-19.4)	86 (83-90)	65 (48-82)

The operating characteristics show the performance of the [TIMP-2]•[IGFBP7] test for discriminating Topaz critically ill subjects with AKI (N = 71) from those with no AKI (N = 337) (see Figure 1 in the manuscript for study design). [a]Sensitivity, specificity and likelihood ratios are operating characteristics that are independent of prevalence of the endpoint (AKI), and therefore provide the most consistent estimation of test performance that can be expected across different patient cohorts that may have different prevalence of AKI. [b]Negative and positive predictive values depend on the prevalence of the endpoint (AKI) and can be calculated from the test sensitivity and specificity and the prevalence. These numbers should be adjusted for the expected prevalence if applied to populations with substantially different prevalence.

Table E5. Summary of prospective studies of urinary biomarkers of acute kidney injury in critically ill patients published before November 2013.

Urinary Biomarker	Study	AUC (95% CI)	End point	Patients/events	Patient population
[TIMP-2]•[IGFBP7]	Bihorac	0.82 (0.76–0.88)	Expert adjudicated AKI	408/71	Mixed ICU
	Kashani[E5]	0.80 (0.76–0.83)	RIFLE I and F	728/101	Mixed ICU
NGAL	Siew[E9]	0.71 (0.63–0.78)	AKIN AKI	451/64	Mixed ICU
	Makris[E10]	0.98 (0.82-0.98)	RIFLE AKI	31/11	Multi-trauma patients in ICU
	Metzger[E11]	0.54 (-)	AKIN AKI	20/9	Mixed ICU
	Martensson[E12]	0.86 (0.68–1.0)	AKIN/RIFLE AKI	45/18	Septic patients in ICU
	De Geus[E13]	0.77 (± 0.05)	RIFLE AKI	632/171	Mixed ICU
	Endre[E14]	0.68 (0.56–0.80)	RIFLE AKI	381/27	Mixed ICU
	Kokkoris[E15]	0.74 (0.64–0.82)	RIFLE AKI	100/36	Mixed ICU
	Doi[E16]	0.69 (0.63–0.75)	RIFLE AKI	339/131	Mixed ICU
	Kashani[E5]	0.71 (0.66 – 0.76)	RIFLE I and F	728/101	Mixed ICU
KIM-1	Metzger[E11]	0.71 (-)	AKIN AKI	20/9	Mixed ICU
	Endre[E14]	0.64 (0.52–0.76)	RIFLE AKI	381/27	Mixed ICU
	Kashani[E5]	0.69 (0.63 – 0.75)	RIFLE I and F	728/101	Mixed ICU
CyC	Nejat[E17]	0.70 (0.64–0.75)	AKIN AKI	444/125	Mixed ICU
	Endre[E14]	0.63 (0.51–0.74)	RIFLE AKI	381/27	Mixed ICU
	Royakkers[E18]	0.49 (-)	RIFLE AKI	151/35	Mixed ICU
IL-18	Endre[E14]	0.72 (0.61–0.83)	RIFLE AKI	381/27	Mixed ICU
	Metzger[E11]	0.57 (-)	AKIN AKI	20/9	Mixed ICU
	Siew[E19]	0.62 (0.54–0.69)	AKIN AKI	451/64	Mixed ICU
	Parikh[E20]	0.73 (-)	AKIN AKI	138/52	Acute lung injury patients in ICU
	Doi[E16]	0.69 (0.62–0.74)	RIFLE AKI	339/131	Mixed ICU
	Kashani[E5]	0.76 (0.71 – 0.81)	RIFLE I and F	728/101	Mixed ICU
L-FABP	Matsui[E21]	0.95 (-)	AKIN AKI	25/11	Mixed ICU
	Kashani[E5]	0.66 (0.60 – 0.72)	RIFLE I and F	728/101	Mixed ICU
	Doi[E16]	0.75 (0.69–0.79)	RIFLE AKI	339/131	Mixed ICU
α-GST	Westhuyzen[E22]	0.89 (0.69–0.97)	ARF	26/4	Mixed ICU
	Walshe[E23]	NR[a]	AKIN AKI	38/19	Septic patients in ICU
π-GST	Westhuyzen[E22]	0.93 (0.74–0.99)	ARF	26/4	Mixed ICU
	Walshe[E23]	~0.5 (-)[a]	AKIN AKI	38/19	Septic patients in ICU
	Kashani[E5]	0.65 (0.60 – 0.71)	RIFLE I and F	728/101	Mixed ICU
NAG	Westhuyzen[E22]	0.84 (0.64–0.95)	ARF	26/4	Mixed ICU
	Matsui[E21]	0.63 (-)	AKIN AKI	25/11	Mixed ICU

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	Doi[E16]	0.62 (0.56–0.68)	RIFLE AKI	339/131	Mixed ICU
GGT	Westhuyzen[E22]	0.95 (0.79–0.99)	ARF	26/4	Mixed ICU
	Endre[E14]	0.61 (0.49–0.73)	RIFLE AKI	381/27	Mixed ICU
	Blasco[E24]	0.86 (0.78–0.95)	25% decrease in CrCl	100/36	Mixed ICU
AP	Westhuyzen[E22]	0.86 (0.68–0.97)	ARF	26/4	Mixed ICU
	Endre[E14]	0.64 (0.52–0.76)	RIFLE AKI	381/27	Mixed ICU
GGTxAP index[b]	Endre[E25]	0.63 (0.52–0.75)	RIFLE AKI	345/52	Mixed ICU

Review of literature additionally included one meta-analysis and five in-depth reviews. [E26-E31]

Abbreviations. TIMP-2, tissue inhibitor of metalloproteinases-2; IGFBP7, insulin-like growth factor binding protein 7; NGAL, Neutrophil gelatinase-associated lipocalin; KIM-1, Kidney injury molecule-1; CyC, Cystatin C; IL-18, Interleukin-18; L-FABP, Liver fatty acid binding protein; α-GST, Alpha-glutathione s-transferase; π-GST, pi-glutathione s-transferase; NAG, N-acetyl-β-D-glucosaminidase; GGT, Gamma glutanyl transpeptidase; AP, alkaline phosphatase.

[a] Exact AUC nor reported, estimate provided.

[b] Index is defined as product of GGT and AP > 46.3 units (indexed to urinary creatinine; dimensionless)

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